

Claims Amended May 2001 for Application Serial No. 08/903,944

Clean Version of Amended Claims

6. (Three Times Amended) A method for producing transgenic poinsettia plants, comprising:
- (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus on auxin- and cytokinin-containing callus induction medium;
- (b) culturing reddish epidermal callus on embryo induction medium comprising casein hydrolysate and  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  to form embryogenic callus;
- (c) (i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or (ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene; wherein the vector or vectors are introduced into the incubating embryogenic callus by co-incubating the callus with *Agrobacterium tumefaciens* containing the vector or vectors or by microprojectile-mediated delivery of the vector into the callus;
- (d) culturing said transformed embryogenic callus on selection medium;
- (e) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
- (f) culturing said transgenic embryos on maturation medium; and

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(g) recovering transgenic plants from said transgenic embryos.

39. (Four Times Amended) A method for producing transgenic poinsettia plants, comprising:

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- (a) incubating poinsettia plant tissue explants that produce reddish epidermal callus in auxin- and cytokinin-containing callus induction medium;
  - (b) subculturing embryogenic callus produced on said callus induction medium to liquid  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$  containing embryo induction medium;
  - (c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;
  - (d) filtering the culture and culturing the filtrate on solid embryo induction medium;
  - (e) subculturing embryos produced on said embryo induction medium to maturation medium;
  - (f) culturing said embryos on callus induction medium;
  - (g) culturing epidermal callus produced on said callus induction medium on embryo induction medium to form embryogenic callus;
  - (h)
    - (i) introducing an expression vector into said embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
    - (ii) introducing two expression vectors into said embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene; wherein the vector or vectors are introduced into the incubating embryogenic callus by
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co-incubating the callus with *Agrobacterium tumefaciens* containing the vector or vectors or by microprojectile-mediated delivery of the vector into the callus;

- (j) culturing said transformed embryogenic callus on selection medium;
- (j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
- (k) culturing said transformed embryos on maturation medium; and
- (l) recovering transgenic plants from said transgenic embryos.

103. (Amended) A method for producing transgenic poinsettia plants comprising the steps of:

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- (a) incubating poinsettia plant tissue explants that produce epidermal callus on auxin- and cytokinin-containing callus induction medium;
  - (b) subculturing embryogenic callus produced on said callus induction medium to liquid embryo induction medium comprising casein hydrolysate and  $\text{NH}_4^+$  and/or  $\text{NO}_3^-$ ;
  - (c) filtering the culture and culturing the filtrate in fresh liquid embryo induction medium;
  - (d) filtering the culture and culturing the filtrate on solid embryo induction medium;
  - (e) subculturing embryos produced on said embryo induction medium to maturation medium;
  - (f) culturing said embryos on callus induction medium;
  - (g) culturing embryogenic callus produced on said callus induction medium on embryo induction medium to form embryogenic callus containing embryos;
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- (i) introducing an expression vector into said incubating embryogenic callus to produce transformed embryogenic callus, wherein said expression vector comprises a selectable marker gene and a second foreign gene, or
  - (ii) introducing two expression vectors into said incubating embryogenic callus to produce transformed embryogenic callus, wherein one of said expression vectors comprises a selectable marker gene, and wherein the second of said expression vectors comprises a second foreign gene;

- (i) culturing said transformed embryogenic callus on selection medium;
  - (j) culturing said transformed embryogenic callus containing embryos on developmental medium containing an osmotic pressure increasing agent;
  - (k) culturing said transformed embryos on maturation medium; and
  - (l) recovering transgenic plants from said transgenic embryos.
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